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14. ABSTRACT

The objective of this work is to develop methodologies for the optimization of field-deployable optical biosensors. We used these Defense University Research Instrumentation Program funds to purchase a Perkin-Elmer GeneAmp PCR System 2400 thermal cycler and a SpectraMax Plus plate reader from Molecular Dynamics. We used the plate reader to develop faster assays for characterizing carbonic anhydrase (CA) variants. We used the thermal cycler to prepare a large library of CA variants. We then completed multiple rounds of selection for variants with enhanced zinc specificity using phage display. We successfully prepared variants with altered metal specificities using these methods. These variants can be used to optimize a carbonic anydrase-based metal ion biosensor.

15. SUBJECT TERMS

Thermal cycler, plate reader, DNA libraries, binding, phage display

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Carol A. Fierke
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FINAL REPORT

Grant#: N00014-97-1-0431

PRINCIPAL INVESTIGATOR: Dr. Carol Ann Fierke

INSTITUTION: University of Michigan (current)
Duke University Medical Center (previous)

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GRANT TITLE: Optimization of Biosensors by Directed Evolution

AWARD PERIOD: 01 March 1997 - 28 February 1998

OBJECTIVE: This Defense University Research Instrumentation Program provides equipment funds for research and education. The objective of this work is to develop methodologies for the optimization of field-deployable optical biosensors. In particular, a carbonic anhydrase-based fiber optic metal ion biosensor will be enhanced by the preparation and use of enzyme variants.

APPROACH: We propose to buy a thermal cycler to facilitate the use of the polymerase chain reaction to amplify DNA for preparing CA variants. Furthermore, we also propose to buy a microplate reader to speed up characterization of CA variants, including the zinc affinity, metal ion specificity, and stability.

ACCOMPLISHMENTS: *Thermal cycler:* We purchased a Perkin-Elmer GeneAmp PCR System 2400 thermal cycler to facilitate the preparation of libraries of CA variants and subcloning of these variants. We prepared a large library ($\approx 10^9$) of CA variants with substitutions at amino acids near the zinc binding site. This library includes substitutions in the direct metal ligands (H94, H96 and H119), the "indirect" metal ligands (Q92, E117 and T199) and the hydrophobic pocket beneath the zinc binding site (F93, F95, W97, L118 and L120). We have previously shown that each of these elements affect the metal binding site, including metal affinity, metal equilibration kinetics and metal specificity. We developed methods to use sulfonamide affinity chromatography in the presence of various Zn/metal ratios to screen this phage library for variants with altered metal ion specificity. We completed multiple rounds of selection for variants with enhanced zinc specificity.

Plate reader: We have purchased the SpectraMax Plus plate reader from Molecular Dynamics with path check capabilities, a monochromator and the ability to measure absorbance in a cuvette. We have used this instrument to assay esterase activity of CAII and the catalytic activity of other enzymes.

We are continuing to work on the development of assays to measure metal ion specificity. Using our conventional methodology for assaying variants, we have determined metal ion affinities and catalytic activity for variants in both the histidine ligands and the hydrophobic pocket beneath the zinc binding site. These data indicate that it is possible to alter the metal ion specificity of carbonic anhydrase.

CONCLUSIONS: We demonstrated that the phage display methodology could successfully be used to identify carbonic anhydrase variants with altered metal specificities.

SIGNIFICANCE: The development of technology to rapidly prepare and screen CA variants for useful properties will significantly enhance the optimization a CA-based metal ion biosensor by increasing the number of variants that can be examined. These methodologies should also be useful for the screening and characterization of any large library.

PATENT INFORMATION: NONE

AWARD INFORMATION: NONE

PUBLICATIONS AND ABSTRACTS (for total period of the grant):

1. Lesburg, C. A., C.-c. Huang, D. W. Christianson, and C. A. Fierke. 1997. Histidine→Carboxamide Ligand Substitutions in the Zinc Binding Site of Carbonic Anhydrase II Alter Metal Coordination Geometry but Retain Catalytic Activity. Biochemistry 36:15780-15791.

2. Hunt, J. A. and C. A. Fierke. 1997. Selection of Carbonic Anhydrase Variants Displayed on Phage: Aromatic Residues in Zinc Binding Site Enhance Metal Affinity and Equilibration Kinetics. J. Biol. Chem. 272:20364-20372.

3. Thompson, R. B., B. P. Maliwal, and C. A. Fierke 1998. Expanded Dynamic Range of Free Zinc Ion Determination by Fluorescence Anisotropy. Anal. Chem. 70: 1749-1754.

4. Thompson, R. B., B. P. Maliwal, V. L. Feliccia, C. A. Fierke, and K. M. McCall. 1998. Determination of picomolar concentrations of metal ions using fluorescence anisotropy: biosensing with a "reagentless" enzyme transducer. Anal. Chem.70: 4717-4723.

5. Hunt, J. A., M. Ahmed and C. A. Fierke. 1999. Metal Binding Specificity in Carbonic Anhydrase is Influenced by Conserved Hydrophobic Core Residues. Biochemistry 38: 9054-9060.

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7. Thompson, R. B., B. P. Maliwal, and C. A. Fierke. 1999. Selectivity and sensitivity of fluorescence lifetime-based metal ion biosensing using a carbonic anhydrase transducer. Anal. Biochem 267: 185-195.

8. Hunt, J. A., M. Ahmed and C. A. Fierke. 1999. Metal Binding Specificity in Carbonic Anhydrase is Influenced by Conserved Hydrophobic Core Residues. Biochemistry 38: 9054-9060.

9. Hunt, J. A., C. A. Lesburg, D. W. Christianson, R. B. Thompson, and C. A. Fierke. 2000. Active Site Engineering of Carbonic Anhydrase and its Application to Biosensors, The Carbonic Anhydrases: New Horizons (Chegwidden, W. R., Carter, N. D. and Edwards, Y. H., ed.). Birkhauser Verlag, Basel/Switzerland. 221-240.

10. Cox, J. D., J. A. Hunt, K. M. Compher, C. A. Fierke and D. W. Christianson. 2000. Structural Influence of Hydrophobic Core Residues on Metal Binding and Specificity in Carbonic Anhydrase II. Biochemistry 39: 13687-13694.